

Synopsis for thesis entitled, "Evaluation of early pathogenic mechanisms of synaptic dysfunction in Alzheimer's disease":

Alzheimer's disease is a debilitating, progressive neurodegenerative disorder in the elderly, characterized by severe loss of memory and higher cognitive functions. In the hundred years since its discovery, Alzheimer's disease (AD) has traversed from the status of a 'rare neurological oddity' to one of the greatest challenges faced by healthcare and medicine in this millennium. A reported 44 million people currently suffer from AD but only 1 in 4 people have been diagnosed. Although AD has been an area of intense research for almost 50 years now, most studies have focused on the end stage disease. Years of study on the pathological cause underlying AD, have conclusively shown that the accumulation of the sticky peptide, $A\beta$, is one of the major triggers of AD pathogenesis. However, after the initial $A\beta$ trigger, multiple processes contribute to disease progression, so that by the time a patient is diagnosed on the basis of overt behavioral phenotypes, it is difficult to understand and differentiate between the causative mechanisms and the consequential effects of the disease. It is, perhaps, because of this, that we are still struggling to find therapies for AD which will stop or at the very least slow the course of the disease. In the 2015 report on AD, issued by the Alzheimer's association, much emphasis has been placed on the early diagnosis of AD and the revision of the diagnostic criteria for AD. According to the new guidelines proposed in 2011, AD has been divided into three stages where the first stage occurs before the appearance of overt behavioral symptoms such as memory loss, whereas by the 1984 guidelines, cognitive disabilities must have already occurred for diagnoses of AD. This proposed preclinical stage of AD has been defined, reflecting the current belief that AD pathogenesis begins almost 20 years before the occurrence of behavioral dysfunction. However, no diagnostic criteria are currently available to establish this stage. Hence, there is a need to understand the early pathogenic mechanisms of AD, which will yield early therapeutic targets as well as early diagnostic markers of AD.

One of the earliest documented events in AD pathogenesis is synaptic dysfunction, which is later manifested as loss of dendritic spines. Deficits in long term potentiation (LTP) has been demonstrated in $A\beta$ exposed hippocampal slices as well as in mouse models of AD, much before the appearance of pathological hallmarks such as plaques and tangles as well as overt behavioral phenotypes. While these and other studies indicate

clearly that elevated levels of soluble A β peptide leads to impairment of synaptic function, the underlying molecular mechanisms are yet to be elucidated. One of the purported mediators of A β induced dysfunction is oxidative stress. The A β peptide, especially the A β 42, is a self aggregating peptide with a propensity to form peptidyl radicals. Interaction of the peptidyl radicals with biomolecules leads to the generation of more free radical species via cascading chain reactions. Additionally, A β peptide has also been demonstrated to have synaptotoxic effects via its effect on NMDA receptors and calcium influx leading to deregulated reactive oxygen species (ROS) production as well as excitotoxicity.

Hence, with a view to understanding A β mediated early synaptic dysfunction in AD, we studied early signaling changes in the synaptosomes derived from the cortex of APP/PS1 mice model of AD at various ages. The APP/PS1 model contains a mouse/human chimeric APP gene bearing the KM670/671NL Swedish mutation and the human PS1 gene with an exon 9 deletion. These mice exhibit behavioral deficits from 7 months of age while plaque deposition and gliosis become apparent by 9 months of age. We chose to study both pre-symptomatic ages (1 and 3 months old) as well as post symptomatic (9 months old) mice. Post nuclear supernatant (PNS) as well as synaptosomes were isolated from the cortex of APP/PS1 and age matched control mice. We assayed the levels of reactive oxygen species (ROS) in the PNS and the synaptosomes of post symptomatic 9 months old APP/PS1 mice and age matched controls. In contrast to reports of enhanced oxidative stress markers in the brains of AD patients, we did not find any increase in the levels of ROS in the PNS of post symptomatic APP/PS1 mice compared to age matched controls. However, synaptosomes from the cortex of these animals exhibited a significant increase in ROS levels in APP/PS1 mice compared to controls. We further found that there was significant increase in the ROS levels in synaptosomes, but not PNS, of very young asymptomatic 1 and 3 months old APP/PS1 mice. This is a first demonstration of synapse specific increase in oxidative stress in AD mice, as young as 1 month of age, indicating that disease specific mechanisms operate at the synapse much before the appearance of any overt cellular or behavioral symptoms. The increase in synaptic ROS levels correlated with a small but significant increase in the levels of A β 42 in the brains of APP/PS1 mice compared to controls. We also found a concurrent change in the redox status of the cytoskeletal protein, actin, at the synapse. As early as 1 month of age, there was a significant decrease in the protein level of reduced actin indicating that there is an increase in the level of oxidized

actin at the synapse. This loss of reduced actin was specific to the fibrillar pool of actin while no significant change was observed in the redox status of the monomeric globular pool of actin. Oxidation of actin has been demonstrated to lead to its depolymerization. Concurrently, we found a significant loss of fibrillar actin in the synaptosomes of APP/PS1 mice. Actin is the major cytoskeletal protein at the synapse. Changes in the globular to fibrillar actin ratio at the synapse at early pre-symptomatic ages in APP/PS1 mice will likely lead to structural and consequent functional changes at the synapse. This could potentially be one of the triggers of synaptic dysfunction in AD.

Furthermore, changes in the Akt-mTOR signaling pathway was also observed in the synaptosomes of 1 month old APP/PS1 mice, which is sustained at 9 months. There was a significant loss of the mTOR-pS6K-4EBP1 axis in the synaptosomes, but not PNS, of APP/PS1 mice. We found that loss of Akt signaling, as evinced by loss of Akt phosphorylation, Akt kinase activity as well as loss of phosphorylation of downstream effector GSK3 β , potentially underlies the loss of mTOR signaling. Further, the loss of Akt signaling is mediated by synapse specific redox modification of Akt and consequent interaction with the protein phosphatase PP2a. Loss of the Akt-mTOR signaling at the synapse is indicative of deficits in local protein translation. Loss of this essential synaptic function, which plays critical roles in synapse maintenance as well as synaptic plasticity during learning and memory, at an early age, will have long ranging impact on synaptic function such as long term potentiation (LTP) in APP/PS1 mice.

Our study is the first demonstration of oxidative stress and consequent signaling changes which occur specifically at the synapse of very young 1 month old APP/PS1 mice. These changes occur much before the appearance of overt phenotype such as plaque deposition and behavioral dysfunction but sustain till the appearance of classical pathological hallmarks. Hence, the study demonstrates that disease progression starts much before previously thought and provides us a critical time window during which therapeutic strategies designed to delay or stop these changes might change the course of AD.